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Poster Session V

Immunology, vaccination and host defences

**DEVELOPMENT OF HYBRID POLYPEPTIDES AS POSSIBLE VACCINE CANDIDATES AGAINST STREPTOCOCCUS AGALACTIAE**

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**Objectives:** *Streptococcus agalactiae*, group B streptococcus (GBS) is a major cause of pregnancy complications and newborn meningitis, pneumonia and sepsis. In our previous study a number of recombinant polypeptides based on surface GBS proteins have been produced and examined for their immunogenic and protective properties. Moreover, the advantage of using the polypeptide complex has been demonstrated. The goal of the present work was to obtain and characterize hybrid polypeptides possessing antigenic epitopes of four polypeptides investigated earlier.

**Methods:** DNA fragments were cloned into pQE 30,32 (Qiagen, USA) and expressed in *E.coli* M15. Ni sepharose (GE Healthcare, Sweden) was used for polypeptide purification. Anti-Scp1a and anti-Scp3a rabbit sera were obtained during rabbit immunization with Scp1a and Scp3a. Monoclonal antibodies were received against N-terminal part of Bac (P7) and N-terminal part of SspB1 (alpha). Scp1-3a was administered subcutaneously in female rabbits (2,5 kg) with alum adjuvant (Thermo, USA) thrice. Immunogenicity was evaluated by ELISA using anti-rabbit IgG conjugated with HRP (Sigma, USA).

**Results:** DNA fragments encoding recombinant polypeptides based on Bac, SspB1, ScpB were obtained by PCR. The resulting DNA fragments carried restriction sites that allowed to produce hybrid DNA fragments followed by cloning. After expression and purification two hybrid polypeptides were obtained. The first one designated as Scp1-3a (M.M. = 17 kDa) contained N-terminal and central parts of ScpB. The second one designated as p7-alpha (M.M. = 80 kDa) contained N-terminal parts of Bac and SspB1. The functional activity of antigenic polypeptide epitopes was proved in ELISA using anti-Scp1a and anti-Scp3a rabbit sera or monoclonal antibodies. Scp1-3a was demonstrated to induce specific IgG during rabbit immunization with maximum titer of  $1:5,12 \times 10^5$ . The rabbit antisera were tested for their opsonizing ability against GBS strains of II, III, V serotypes and GAS strain of M49 serotype employing mouse peritoneal macrophages. The immune rabbit antisera were found to reveal the protective activity more than twice compared with normal rabbit sera against all streptococcal strains studied. The advantage of the new hybrid polypeptides is discussed.

**Conclusion:** The study has demonstrated that the new hybrid polypeptides composed of different parts of Bac, SspB1, ScpB possessed functionally active epitopes and could be considered as vaccine candidates against *Streptococcus agalactiae*.

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